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JAK's SOCS: A Mechanism of Inhibition

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SOCS1 and SOCS3 are specific inhibitors for JAK tyrosine kinases. In this issue of *Immunity*, Babon et al. (2012) discovered the inhibition mechanism of SOCS3 by employing nuclear magnetic resonance and classical enzyme kinetics.

Janus kinase (JAK), which is a key signal transmitter of cytokines, has been shown to be an attractive therapeutic target for cancer and inflammatory diseases. Most cytokines, including interleukins, interferons (IFNs), and hematopoietic growth factors, activate JAKs. In mammals, the JAK family comprises four members: JAK1, JAK2, JAK3, and TYK2. JAK1, JAK2, and TYK2 appear to be ubiquitously expressed, whereas JAK3 expression is normally limited to hematopoietic cells. Activated JAKs phosphorylate the associated receptor cytoplasmic domains, which then creates docking sites for SH2-containing signaling proteins, including signal transducers and activators of transcription (STATs). The Ras-ERK and PI3 kinase pathways are also activated through JAKs. Aberrant activation of these pathways is often observed in many cancer and leukemic cells. Hyperactivation of the JAK-STAT pathway plays a role in several immunological disorders such as inflammatory diseases, autoimmune diseases, and allergy.

Several JAK2 inhibitors are under development for the treatment of myeloproliferative neoplasias (MPN) and other tumors. This is because constitutive activation of JAK2 was found in leukemias and lymphomas (via formation of chimeric proteins) and in MPN (via a V617F mutation). JAK inhibitors are also effective for tumors with constitutive JAK-STAT pathway activation without mutations. Therapeutic benefit from JAK kinase inhibition has already been established in rheumatoid arthritis (RA) with the use of CP-690,550 (Tofacitinib), a pan-JAK inhibitor. CP-690,550 was originally intended for organ transplantation immunosuppression because it is a potent inhibitor of JAK3, but has also shown to have activity against JAK1 and JAK2. More recently, the selective JAK1, JAK2 inhibitor, INCB028050, has demonstrated efficacy in various rodent models of RA, further demonstrating the central role JAK kinases play in this disease.

There is a natural inhibitor family for the JAKs: the SOCS family of proteins (Alexander and Hilton, 2004; Yoshimura et al., 2007). Overexpression of these proteins has been shown to effectively suppress tumors and RA models (Yoshimura et al., 2007). Thus, SOCS mimetics is a strategy for developing therapeutics to these diseases. However, a precise mechanism of how SOCS inhibits JAK kinase activity remains to be established. In this issue of *Immunity*, Babon et al. (2012) succeeded in obtaining NMR spectrums of JAK2 kinase domain and the SOCS3 complex, and, in combination with classical biochemical enzyme assays, they discovered an unexpected mechanism through which SOCS3 inhibits JAK kinase activity.

The suppressor of cytokine signaling (SOCS) protein family comprises eight members (cytokine-inducible SH2 protein [CIS] and SOCS1–SOCS7). The central SH2 domain determines the target of each SOCS and CIS protein. There is a conserved sequence called extended SH2 domain (ESS) adjacent to the SH2 domain, which is necessary for a high-affinity binding of the SH2 domain to the target phosphopeptides (Babon et al., 2006; Yasukawa et al., 1999). The SH2 domain (including ESS) of SOCS1 directly binds to the activation loop of JAK (Yasukawa et al., 1999). While the SH2 domains of CIS, SOCS2, and SOCS3 bind to phosphorylated tyrosine residues on activated cytokine receptors, SOCS3 binds to gp130-related cytokine receptors, including the phosphorylated tyrosine 757 (Y757) residue of gp130, the Y800 residue of IL-12 receptor β2, and Y985 of the leptin receptor, showing that suppression by SOCS3 is relatively specific to STAT3 and STAT4. SOCS3 does not inhibit IL-10-mediated STAT3

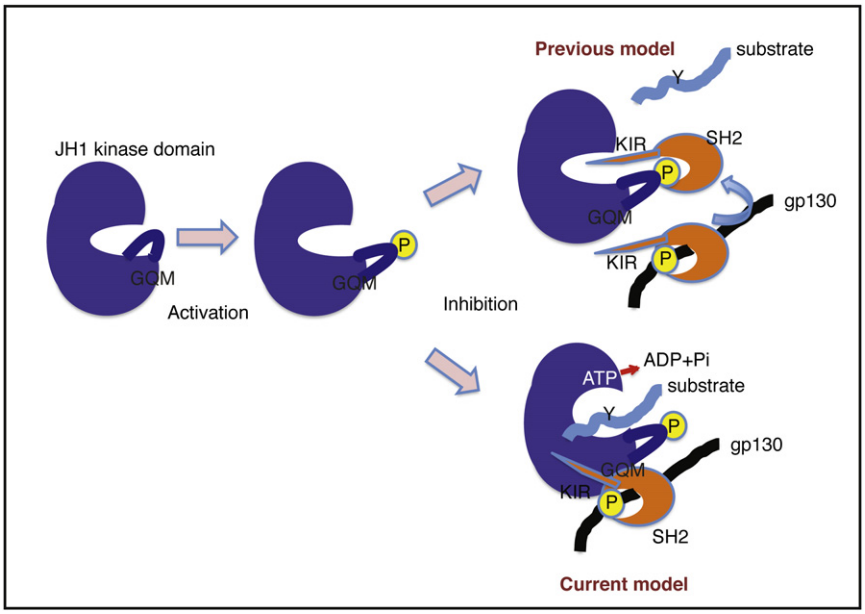

Figure 1. Current Model of the Inhibition of JAKs by SOCS3
In a previous model, the KIR domain of SOCS3 (orange) functioned as pseudosubstrate for JAK kinase (purple). In the current model proposed by Babon et al. (2012), based on a structural analysis of the complex, SOCS3 binds to the surface of the JH1 domain, which contains a GQM motif and induces a conformational change of the catalytic pocket, so as to block transfer of a phosphate group to the substrate. KIR is involved in the binding of SOCS3 to JH1.

activation because SOCS3 does not bind to the IL-10 receptors (Yasukawa et al., 2003). This is an important mechanism for IL-6 and IL-10 to exhibit different immunological effects.

This family has a conserved carboxy-terminal 40 amino acid module known as the SOCS box. The SOCS box interacts with elongin B and elongin C and other molecules that recruit E2 ubiquitin transferase (Babon et al., 2009). Thus SOCS family proteins, as well as other SOCS-box-containing molecules, function as E3 ubiquitin ligases and mediate the degradation of proteins that are associated with these family members through their N-terminal regions.

In addition to their ability to suppress signaling by ubiquitin-mediated degradation of the signaling complex, both SOCS1 and SOCS3 are found to inhibit JAK tyrosine kinase activity directly, probably through their kinase inhibitory region (KIR). KIR is composed of 12 amino acids and has been proposed to function as a pseudosubstrate that is essential for the suppression of cytokine signals (Yasukawa et al., 1999). This model is based on the following facts. (1) KIR is essential for a high-affinity binding of SOCS1 and SOCS3 to the kinase domain (so called JH1) of JAKs, but not necessary for binding to the peptides containing phosphorylated tyrosine residues. (2) KIR peptide contains a tyrosine residue that is a good substrate of JAKs in vitro, but is never phosphorylated in vivo. (3) A high concentration of KIR peptide inhibits JAK kinase activity, which is apparently due to competitive inhibition with substrates. However, other possibilities such as direct inhibition of JH1 by the KIR peptide (or by its modified peptides) have been proposed (Doti et al., 2011). Thus, the precise mechanisms of suppression of kinase activity by KIR remain to be clarified.

It has not been clear how SOCS3 inhibits JAK kinase after binding to gp130, despite a low affinity of KIR peptide to JH1. Because the whole SOCS3 (SH2-domain+ESS+KIR) molecule can bind to JH1 with high affinity, we proposed that SOCS3 binds to the receptors first, then moves to the kinase domain by interacting with the phosphorylated activation loop though the SH2 domain, and then KIR interacts with catalytic pocket (Figure 1; Sasaki et al., 1999; Yoshimura et al., 2007). A similar mechanism has been considered for SOCS1; it binds to the IFN-γ receptor (IFNGR1) first, then binds to JAK2 and inhibits kinase activity. However, it is still unknown how KIR inhibits kinase activity.

To understand precise mechanism of suppression of JAKs by SOCS1 and SOCS3, X-ray or NMR structural analysis of JAK and SOCS complex has been anticipated. Previously, the X-ray crystal structures of SOCS3 with gp130 phosphopetptide and SOCS2 with the ElonginB-C complex were resolved (Babon et al., 2006, 2009). Although these studies defined the structures necessary for the interaction with ElonginB-C or stability of SOCS3, the function of KIR could not be elucidated.

By using NMR spectrums of JAK2-JH1 and the SOCS complex in combination with biochemistry, Babon et al. (2012) now reveal how SOCS3 inhibits JAK kinase. First, they show that SOCS3 binds and directly inhibits the catalytic domains of JAK1, JAK2, and TYK2, but not JAK3. JAKs 1 and 2 and TYK2 (but not JAK3) possess an evolutionarily conserved motif unique to JAKs, a GQM motif in the JAK insertion loop. This motif is not the entire binding surface on JAK-JH1, but is an essential motif within the interaction surface between JH1 and SOCS3. Importantly, the gp130 phosphopeptide induces a conformational change of the KIR-ESS-SH2 domain of SOCS3 so that SOCS3 can bind to the surface of JH1. Then, kinetic experiments showed that SOCS3 is a *noncompetitive* inhibitor of JAK2-JH1 with respect to both ATP and substrate, and that SOCS3 actually increases ATPase activity of JH1. The authors propose that SOCS3 specifically inhibits the ability of JH1 to transfer phosphate to tyrosine but does not inhibit its ability to hydrolyze ATP, and thus increase the transfer of phosphate to water (Figure 1).

This is a surprising and unique mechanism we never expected. All other known protein kinase inhibitors act by competitive mechanisms, either by inhibiting ATP or substrate binding directly. Their model can explain a highly specific inhibition mechanism for JAK (except for JAK3) by SOCS3 after binding to the receptor. Because current JAK inhibitors are all ATP analogs, this information will provide clues to develop novel types of JAK inhibitors. Importantly, ATP analog JAK

inhibitors CP-690,550 and Pyridone6 were found to inhibit STAT3 less efficiently than STAT1, STAT, and STAT6 (Yoshida et al., 2012). Thus, at low concentrations, these inhibitors promote T helper 17 (Th17) cell development by suppressing Th1 and Th2 cell development. Unlike in vitro, CP-690,550 seems to inhibit JAK1 much less efficiently than JAK3 in vivo (Yoshida et al., 2012). Thus, new inhibitors specific to the GQM motif with a non-ATP-competitive mechanism could be more specific to JAK1 and may inhibit STAT3 very efficiently. Such a drug may be suitable for treatment of Th17 cell-mediated diseases and cancers where STAT3 plays critical roles.

Even though Babon et al. (2012)'s study is extremely elegant, several questions remain to be solved. First, the interaction points between KIR and JH1 were not clarified in this study because the KIR region of SOCS3 is unstructured in the absence of JAK2-JH1. Cocrystal analysis may be necessary to resolve this problem. Second, it remains to be established whether this mechanism of SOCS3 can be extended to SOCS1. The SOCS1-IFNGR1 complex may inhibit JH1 just like the SOCS3-gp130 complex. However, apparently, the SOCS1 SH2 domain has a high affinity to the activation loop of JH1 and can inhibit almost all cytokine signaling by overexpression in vivo. The interaction of SOCS1 with JH1 may be different from that of SOCS3. The mechanism of SOCS1 is a challenge of structural biology because nobody has been successful in obtaining recombinant soluble and functional SOCS1 protein. Third, the current findings should be translated into drug discovery. In silico structural modeling of JAKs and SOCS may facilitate discovery of new drugs that mimic SOCS.

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Toll-like Receptor 9, What O'Clock Is It?

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In this issue of *Immunity*, Silver et al. (2012) provide evidence that murine Toll-like receptor 9 (TLR9) expression and function in innate and adaptive immunity is controlled by the circadian cycle.

Toll-like receptor 9 (TLR9) belongs to the TLR family of pattern recognition receptors (PRRs) that are important for sensing evading pathogens via conserved pathogen-associated molecular patterns (PAMPs). The family of TLRs consists of 13 members (TLR1 to TLR13) that sense different PAMPs such as lipopolysaccharide, lipoproteins, flagellin, and nucleic acids (Iwasaki and Medzhitov, 2004). TLR9 is part of a subgroup of TLRs that is expressed in endosomal vesicles and recognizes bacterial and viral DNA with a sequence motif containing the dinucleotide CpG. Importantly, ligand sensing by TLR9 leads to subsequent production of type I interferon, proinflammatory cytokines and upregulation of costimulatory molecules, signals essential for initiating and directing an antigen-driven adaptive immune response (Iwasaki and Medzhitov, 2004; Kumagai et al., 2008). Synthetic CpG-containing deoxynucleotides that mimic microbial DNA are undergoing clinical testing as adjuvant in vaccines and as immunomodulator against infection or for the treatment of cancer and allergy (Jurk and Vollmer, 2007). However, under certain circumstances such as enhanced DNA uptake, non-CpG-containing DNA or self-DNA can also activate TLR9 and induce or sustain certain autoimmune diseases such as systemic lupus erythematosus (SLE). Accordingly, self-DNA-antibody complexes trigger TLR9 in SLE and lead to the production of type I interferon, which is involved in pathogenesis of the disease. Thus, TLR9 antagonists are also currently evaluated for therapeutic use, especially for the treatment of SLE (Marshak-Rothstein, 2006).